longer. Recent findings indicate that activation of the SEF can influence the excitability of gaze centres; when monkeys suppress a reflexive saccade to produce an antisaccade, neurons in the SEF show increased activity[24] while neurons in FEF[27] exhibit reduced activity. Also, electrical stimulation of the SEF inhibits neuronal activity in the FEF[28] and can delay movements[29,30]. Thus, diverse observations about SEF function might be accommodated by the hypothesis that the SEF functions as a node in the brain's supervisory control system. □

Methods

Two male macaque monkeys (*Macaca mulatta, Macaca radiata*) were prepared for training and physiological recording using aseptic procedures under isofluorane anaesthesia. The experimental protocol conformed to United States Public Health Service guidelines and was approved by the Vanderbilt Animal Care Committee. A PDP-11/83 presented stimuli and collected eye position, spike and event data.

The application of the eye movement countermanding task in neurophysiological experiments has been described[14]. After a central spot was fixated, it disappeared at the same time as a visual target was presented either in the most sensitive zone of a neuron's response field or in the opposite hemifield at the same eccentricity. On a fraction of trials after a delay, referred to as the stop-signal delay, the fixation spot reappeared, instructing monkeys to withhold the movement ('stop-signal trials'). During the trials in which the stop signal was not presented ('no-stop-signal trials') monkeys were rewarded for generating a single saccade to the peripheral target. During stop-signal trials monkeys were rewarded for maintaining fixation on the central spot ('cancelled trials'). If the monkeys generated a saccade to the peripheral target during stop-signal trials ('non-cancelled trials'), no reward was given. On correct trials juice reward was given on a variable ratio schedule coupled with an acoustic secondary reinforcer given on every trial.

Performance in the countermanding task is probabilistic because of the variability in reaction times across trials. The probability of not cancelling the movement increases as the delay between the signal to initiate the movement and the signal to inhibit the movement ('stop-signal delay') increases. Stop-signal delays were varied according to the monkeys' performance, so that at the shortest (longest) stop-signal delay monkeys generally inhibited the movement on more than 85% (less than 15%) of the stop-signal trials. Movements generated with a short latency tend to be initiated before the stop-signal can influence the system. Conversely, movements generated with long latencies tend to be inhibited because there is enough time for the stop signal to influence the system. The time needed to cancel the movement, known as 'stop-signal reaction time', can be estimated from a simple race model; this model determines the response time on no-signal trials that corresponds to the probability of cancelling a movement at each stop-signal delay. The mean stop-signal reaction time calculated from the behavioural data collected while recording from SEF neurons was 100 ms (A, 104 ms; H, 95 ms).

Neural activity was compared between different types of trials using average activation functions constructed by convolving spike trains with a combination of growth and decay exponential functions that resembled a postsynaptic potential. Criteria for a significant difference in activity between either cancelled or non-cancelled trials and the appropriate latency-matched no-signal trials was that the difference in average firing rate exceed by 2 standard deviations (s.d.) the mean difference in activity during the 600-ms interval before target presentation, provided that the difference reached 6 s.d. and remained above the 2 s.d. threshold for 50 ms. The time interval between the beginning of differential activity and the stop signal reaction time was then determined. The magnitude of modulation in cancelled trials was measured as the time-averaged difference in discharge rate between the activity on cancelled and latency-matched no signal trials.

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High constitutive activity of native H_3 receptors regulates histamine neurons in brain

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Some G-protein-coupled receptors display 'constitutive activity', that is, spontaneous activity in the absence of agonist[1–4]. This means that a proportion of the receptor population spontaneously undergoes an allosteric transition, leading to a conformation that can bind G proteins[3]. The process has been shown to occur with recombinant receptors expressed at high density, and/or mutated, but also non-mutated recombinant receptors expressed at physiological concentrations[5–7]. Transgenic mice that express a constitutively active mutant of the β_2-adrenergic receptor display

cardiac anomalies[8]; and spontaneous receptor mutations leading to constitutive activity are at the origin of some human diseases[9,10]. Nevertheless, this process has not previously been found to occur in animals expressing normal levels of receptor[3,4]. Here we show that two isoforms of the recombinant rat H_3 receptor[11,12] display high constitutive activity. Using drugs that abrogate this activity ('inverse agonists') and a drug that opposes both agonists and inverse agonists ('neutral antagonist'), we show that constitutive activity of native H_3 receptors is present in rodent brain and that it controls histaminergic neuron activity *in vivo*. Inverse agonists may therefore find therapeutic applications, even in the case of diseases involving non-mutated receptors expressed at normal levels.

Starting from the sequence of the human H_3 receptor[13], we screened a complementary DNA library from rat striatum and isolated two full-length complementary DNAs encoding 445- and 413-amino-acid sequences (Fig. 1a) that we termed H_{3L} and H_{3S}, in analogy with the corresponding H_3-receptor variants in guinea-pig[14]. The existence of these variants results from splicing at a level corresponding to the mid-portion of the third intracytoplasmic loop (i3) of the H_3 receptor. Alternative splicing of the dopamine D_2 receptor[15,16] at almost the same level leads to two isoforms with similar pharmacology and only limited differences in G-protein-coupling efficiency. In the case of the H_3 receptor, comparative binding studies on different tissues and the multiphasic competition curves observed with some antagonists has suggested the existence of distinct receptor subtypes[17–19]. The existence of isoforms derived from the same gene with limited pharmacological differences (Table 1) may account partly for these observations, although our polymerase chain reaction (PCR) study shows that the H_{3L} receptor is largely predominant in all tissues (Fig. 1b).

The carboxy terminus of i3 has a stretch of eight amino acids that are highly similar to the corresponding sequence of a mutated human β_2-adrenergic receptor in which the mutation confers a constitutive activity (CAM hβ_2-AR in Fig. 1a) that is absent in the native receptor[20]. Thus, among these eight amino acids, six (five in the mouse) are identical in the rat H_3 receptor and in the mutated β_2-adrenergic receptor, whereas the other two amino acids are conserved. Furthermore, this region is also critical for constitutive activity in other native or mutated heptahelical receptors[5,9,21].

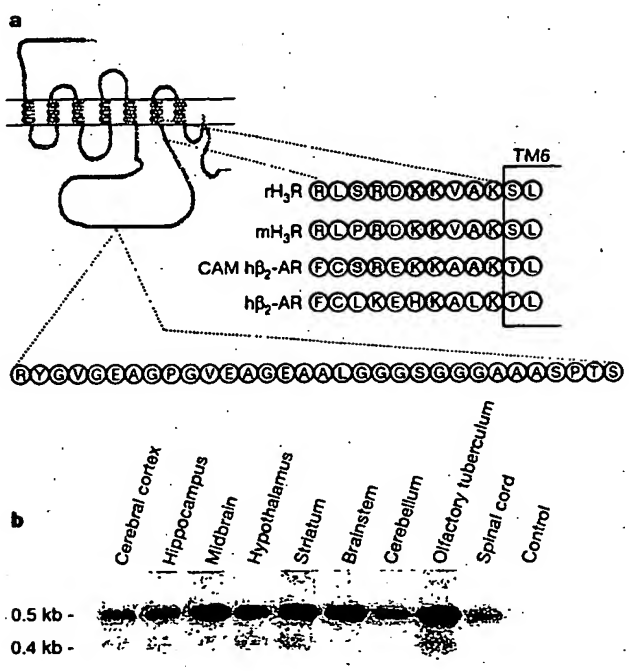

Figure 1 Two rat H_3-receptor isoforms and their expression in rat brain regions. **a**, Putative seven-transmembrane topography of H_{3S} and H_{3L} alternatively spliced variants differing by a 32-amino-acid insertion in the i3 loop. This loop C-terminal sequence is compared with that of the mouse (mH_3R), the native human β_2-adrenergic receptor (hβ_2-AR) and a constitutively active mutant (CAM hβ_2-AR) (ref. 20). **b**, RT–PCR analysis of H_{3S} and H_{3L} receptor RNAs using primers flanking the spliced region

Table 1 Potencies of H_3-receptor ligands in CHO(H_{3S}) and CHO(H_{3L}) cells

Agents	[^{3}H]histamine release K_i or EC_{50} (nM)	[^{3}H]arachidonic acid release K_i or EC_{50} (nM)	
		H_{3S}	H_{3L}
Agonists			
Histamine	200 ± 50*	110 ± 32	141 ± 39
Imetit	1.0 ± 0.3†	5 ± 2	0.4 ± 0.2
Inverse agonists§			
Thioperamide	2.2 ± 0.6	0.3 ± 0.1	0.05 ± 0.03
Ciproxifan	0.5 ± 0.1‡	0.07 ± 0.03	0.11 ± 0.06
FUB 465	580 ± 230	18 ± 6	10 ± 5
Antagonist			
Proxyfan	19 ± 6	5.0 ± 1.7	6.3 ± 2.3

The potencies of H_3-receptor ligands were compared for both [^{3}H]histamine release from depolarized synaptosomes and A23187-evoked [^{3}H]arachidonic acid release from CHO(H_{3S}) and CHO(H_{3L}) cells.
* Data from ref. 26
† Data from ref. 24
‡ Data from ref. 25
§ Values represent the EC_{50} for [^{3}H]arachidonic acid release and the K_i for [^{3}H]histamine release from rat cortical synaptosomes when opposed to histamine in 30 mM K^+ medium

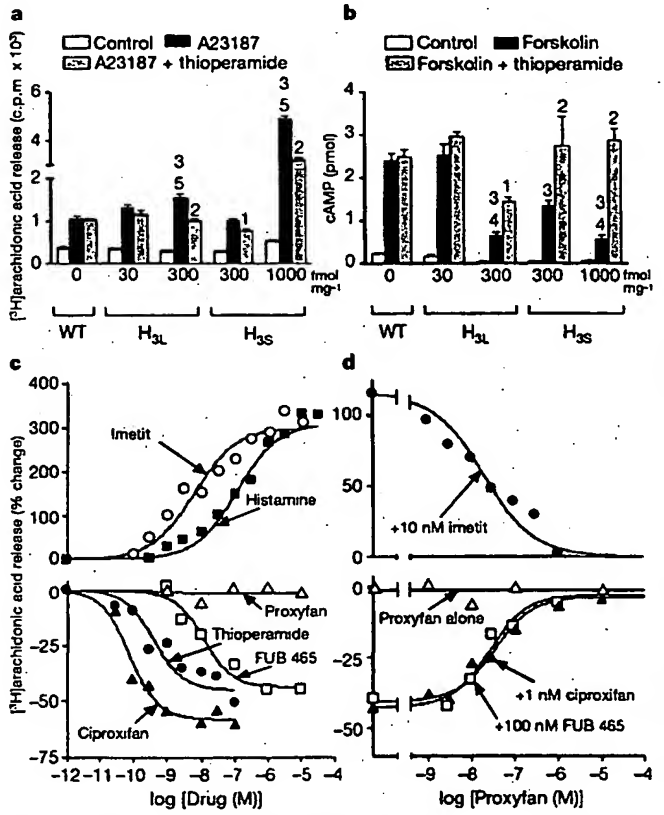

Figure 2 Constitutive activity and pharmacology of H_{3S} or H_{3L} receptors expressed in CHO cells. Effects of thioperamide on A23187-evoked [^{3}H]arachidonic acid release (a) or forskolin-evoked cAMP accumulation (b) in CHO cells expressing various densities of the two isoforms. Results are means ± s.e.m. of 3–10 determinations in an experiment replicated with similar data. $P < 0.05$ (1), $P < 0.001$ (2) versus A23187 or forskolin alone; $P < 0.001$ (3) versus wild-type cells; $P < 0.01$ (4), $P < 0.001$ (5) versus CHO(H_{3S}) cells expressing 300 fmol per mg protein. **c**, **d**, Effects of H_3-receptor ligands on A23187-evoked [^{3}H]arachidonic acid release from CHO(H_{3S}) cells expressing 500 fmol per mg protein. Results are expressed as the per cent change compared with A23187-evoked release (1,308 ± 22 c.p.m. per well).

We have therefore assessed the constitutive activity of the H_{3L} and H_{3S} isoforms expressed in Chinese hamster ovary (CHO) cells at low, medium and high densities: 30–80, 300–500 and ~1,000 fmol per mg protein as determined by [^{125}I]iodoproxyfan assay[22], respectively. The coupling changes associated with receptor expression were evaluated in two signalling pathways activated by histamine and involving G_i/G_o proteins: adenylyl cyclase inhibition and phospholipase A_2 activation. In both pathways, constitutive activity of both the H_{3L} and H_{3S} receptors was clearly evidenced. In addition, [^{3}H]arachidonic acid release evoked by the Ca^{2+}-ionophore A23187 was enhanced, whereas forskolin-induced cyclic AMP accumulation was reduced (in both cases in total absence of histamine) when the receptor density was enhanced; these changes were largely reversed in the presence of thioperamide, a compound so far considered as the prototypical H_3-receptor antagonist[12], but functioning here, as predicted by binding data[23], as an extremely potent inverse agonist (Fig. 2).

Constitutive activity was slightly more pronounced with the H_{3L} isoform: there was a tendency for spontaneous activity and a response to thioperamide at low expression levels of H_{3L} (effects that became significant at 80 fmol per mg protein; data not shown), and, at intermediate levels, changes were more marked than with the H_{3S} isoform (Fig. 2a, b); however, the difference between the isoforms was modest as also shown by the limited difference in potency of the agonist imetit[24] (and the similar potency of histamine). A primary characteristic of the active conformation of G-protein-coupled receptors is their higher affinity for agonists[3].

These observations suggested that constitutive H_3-receptor activity was likely to occur in brain where the H_{3L} isoform predominates, and where the density of [^{125}I]iodoproxyfan-binding sites[22] is presumably more than 500 fmol per mg protein in cells expressing the H_3 receptor (assuming that these cells represent less than 20% of the total). To assess this possibility, we needed to identify ligands displaying well-defined agonist, inverse agonist and neutral antagonist properties in cell lines, and then to determine the effects of these probes at the native cerebral receptor. Neutral antagonists are not

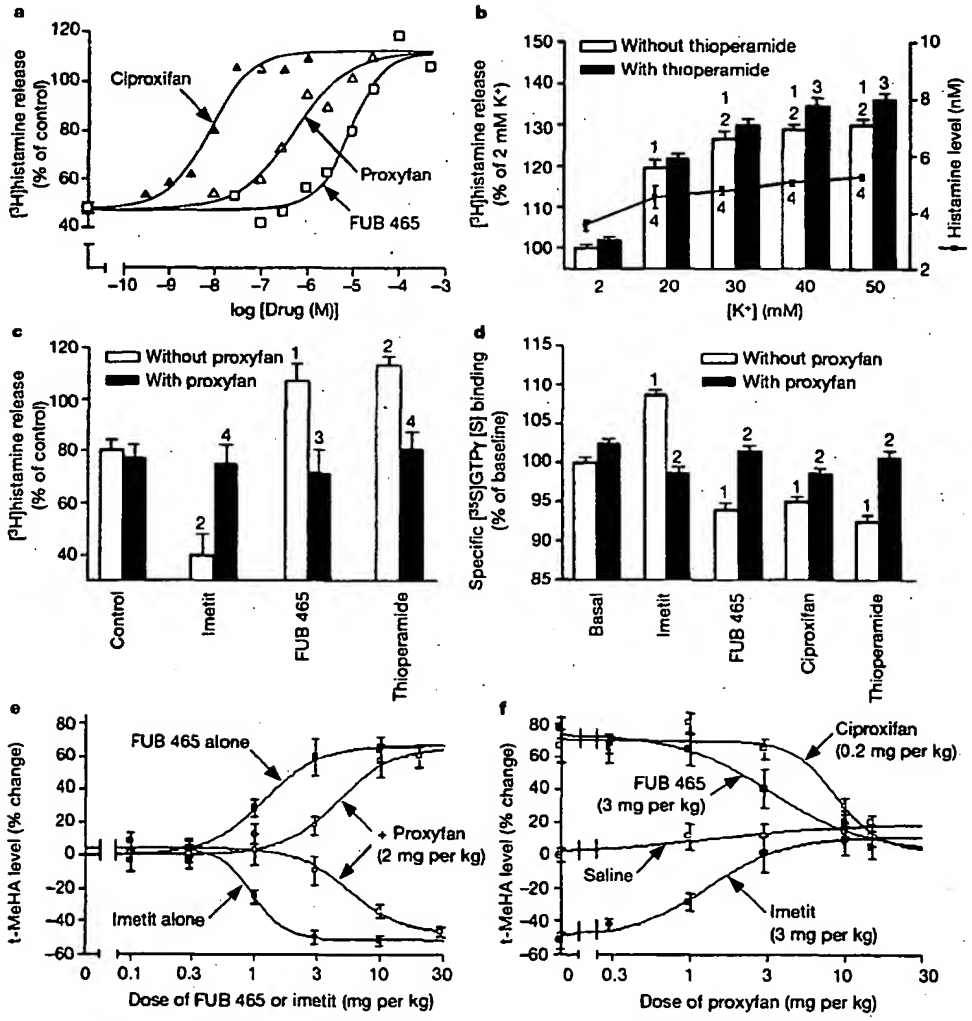

Figure 3 Effects of H_3-receptor ligands on responses mediated by native H_3 receptors in rodent brain. **a**, Reversal of histamine-induced inhibition of [^{3}H]histamine release from rat synaptosomes depolarized by 30 mM K^+. Means from two separate experiments with quadruplicate determinations in each. **b**, Effect of thioperamide on [^{3}H]histamine release from mouse synaptosomes depolarized by increasing K^+ concentrations. Levels of endogenous histamine released in the medium by K^+ were determined. Results are means ± s.e.m. of 13–23 determinations from three separate experiments. $P < 0.001$ (1) versus 2 mM K^+; $P < 0.01$ (2) versus 20 mM K^+; $P < 0.05$ (3) versus without thioperamide; $P < 0.05$ (4) versus 2 mM K^+. **c**, Effects of H_3-receptor ligands on [^{3}H]histamine release induced by 55 mM K^+ from mouse synaptosomes in the presence or absence of proxyfan. Means ± s.e.m. of 12–40 determinations from four separate experiments. $P < 0.01$ (1), $P < 0.001$ (2) versus control; $P < 0.01$ (3), $P < 0.001$ (4) versus without proxyfan. **d**, Effects of H_3-receptor ligands on [^{35}S]GTPγS binding to mouse cerebral cortical membranes in the presence or absence of proxyfan. Results are means ± s.e.m. of 9–24 determinations from four separate experiments. $P < 0.001$ (1) versus basal; $P < 0.01$ (2) versus without proxyfan. **e**, **f**, Changes in brain t-MeHA levels in mice receiving H_3-receptor ligands p.o. Means ± s.e.m. of 8–16 values

easily identified because, theoretically, they correspond to ligands displaying equal affinity for the active and inactive receptor conformations, a condition obviously difficult to achieve[1,4]. We found that a large number of tested antagonists (defined by their ability to block the histamine response at the autoreceptor inhibiting [^{3}H]histamine release from synaptosomes) behaved as potent inverse agonists: they decreased [^{3}H]arachidonic acid release from CHO cells at concentrations up to two orders of magnitude less than those required to antagonize histamine at the H_3 autoreceptor in synaptosomes. This included FUB 465 (ethyl-3-(1*H*-imidazol-4-yl)propyl ether), an antagonist with only micromolar affinity at the synaptosomal autoreceptor (Fig. 3a) and with inverse agonist activity with an effective concentration for half-maximal response (EC_{50}) of ~10 nM in CHO(H_3) cells (Fig. 2c).

In contrast, we identified proxyfan (3-(1*H*-imidazol-4-yl)propylphenylmethyl ether) as a potent (inhibition constant (K_i) ≈ 10 nM) neutral antagonist: first, it inhibited the effects of histamine at the synaptosomal H_3 autoreceptor (Fig. 3a); second, in CHO(H_3) cells with moderate expression, it inhibited those of the agonist imetit and those of the inverse agonists ciproxifan[25] and FUB 465, without affecting [^{3}H]arachidonic acid release alone, even at a 10 μM concentration (Fig. 2d). As expected[1], however, the pharmacological profile of proxyfan depended on the test system; that is, it depended on the equilibrium between the active and inactive conformations of the receptor and/or the stoichiometric ratio of the receptor to the various G proteins. Proxyfan displayed partial inverse agonism on [^{3}H]arachidonic acid release in CHO cells with high expression and partial agonism when cAMP was evaluated (data not shown).

Using these various probes, we could determine the constitutive activity of the H_3 autoreceptor controlling [^{3}H]histamine release in cortical synaptosomes submitted to a strong depolarizing stimulus (40–55 mM K^+) in the mouse (Fig. 3b, c) or rat (data not shown). Both thioperamide and FUB 465 behaved as inverse agonists, enhancing significantly the amine release, release being on the contrary reduced by the agonist imetit. Proxyfan, which alone did not affect [^{3}H]histamine release, blocked the opposite effects of either thioperamide and FUB 465 or imetit, therefore acting again as a neutral antagonist (Fig. 3c). These responses could not involve endogenous histamine, and the endogenous amine level, even in the 55 mM K^+ medium, was two orders of magnitude less than its EC_{50} value as an agonist[26] (5.3 ± 0.2 nM versus 200 ± 50 nM). Therefore blockade of the H_3-autoreceptor stimulation by endogenous histamine does not significantly contribute to the releasing effect of drugs like thioperamide (as we had originally proposed[11,12]). This is also shown by (1) the lack of releasing effect of proxyfan, a potent neutral antagonist, (2) the potent releasing effect of FUB 465, a potent inverse agonist but weak neutral antagonist, and (3) the evidence for constitutive activity in cerebral membranes devoid of histamine using a [^{35}S]GTPγS-binding test.

In agreement, binding of the guanylnucleotide analogue [^{35}S]GTPγS to mouse (or rat; data not shown) cerebral membranes demonstrated the coupling of the native H_3 receptor with G proteins[27], in other words, constitutive activity (Fig. 3d). Thus, specific [^{35}S]GTPγS binding was reduced significantly by FUB 465, ciproxifan or thioperamide, which were acting as inverse agonists as their effects were blocked by 1 μM proxyfan. Proxyfan also blocked the increase in binding elicited by imetit, but did not itself significantly affect binding and was therefore acting again as a neutral antagonist in this test system. In contrast with this pattern, yohimbine, an inverse agonist at overexpressed or mutated α_2-adrenergic receptors, did not decrease [^{35}S]GTPγS binding to cerebral membranes, indicating that constitutive receptor activity is not an inevitable consequence of the experimental conditions required to evaluate the binding, as previously proposed[1].

Moreover, all three inverse agonists markedly enhanced cerebral histamine neuron activity *in vivo*, increasing the levels of the histamine metabolite *tele*-methylhistamine (t-MeHA), a reliable marker of this activity[28] (Fig. 3e, f), by ~80% at maximum, and increasing histamine turnover as evaluated by the pargylin-induced t-MeHA accumulation in brain (not shown). This effect reflects an inverse agonist rather than the antagonist activity (towards endogenous histamine) of these ligands, as has been assumed so far[12,28]. In agreement, the effect of FUB 465 obtained at a low dose (half-maximal effective dose (ED_{50}) ≈ 1 mg per kg per os, p.o.) was more consistent with its nanomolar potency as an inverse agonist than its micromolar potency as an antagonist. This situation is not restricted to FUB 465, and the potency of a large series of ligands in enhancing t-MeHA level in brain is not well correlated with their antagonist potency[29,30], but more so with their inverse agonist potency (X.L., S.M., C.R.G., W.S., J.-C.S. & J.M.A.; manuscript in preparation). Moreover, the increases in t-MeHA level by FUB 465 and ciproxifan were competitively antagonized by proxyfan given at doses of ~2 mg per kg which also blocked the decrease in t-MeHA level induced by the agonist imetit. At these doses, proxyfan administered alone failed to affect significantly levels of t-MeHA, indicating that it was acting as a neutral antagonist *in vivo* on a system regulated by H_3 receptors displaying constitutive activity. The small but significant increase (by ~20%) observed with proxyfan in doses above 10 mg per kg might reflect its antagonist activity towards endogenous histamine.

Activation of histaminergic neurons, which promotes arousal and attention, and improves learning in normal animals, has been proposed as a symptomatic therapeutic approach in human attentional and ageing disorders, such as attention-deficit hyperactivity disorders and Alzheimer's disease[25]. Our observations indicate that such an effect is more likely to be obtained with H_3-receptor inverse agonists rather than with neutral antagonists, as has been assumed so far. □

Methods

Cloning of rat H_3 receptor cDNA isoforms

A rat striatal cDNA library (4×10^6 phages; Stratagene) was screened at high stringency with a ^{32}P-labelled fragment (607 base pairs) obtained by PCR with reverse transcription (RT–PCR) of total RNAs from rat cerebral cortex using primers 1 and 2 (ref. 13). These primers are based on the sequence of the third transmembrane domain and the third intracellular loop of the human H_3 receptor, respectively. Bluescript KS(+) plasmids were recovered from 60 positive clones and their cDNA inserts sequenced. The sequence of the rat H_{3L} and H_{3S}-receptors has been deposited in GenBank (accession numbers AY009370 and AY009371). The full-length mouse H_3-receptor cDNA was obtained by RT–PCR.

RT–PCR analysis

RNAs from various rat brain regions, rat spinal cord (10 μg) and mouse striata (2 μg) were used for first strand cDNA synthesis using avian myeloblastosis virus reverse transcriptase (50 units, Roche) and 0.19 μM random primer p(DN)$_6$ (Roche). These templates were amplified for 30 cycles (94 °C for 30 s and 68 °C for 10 min) using gene Amp XL PCR kit (Perkin–Elmer), and either primers 3 and 4 corresponding to nucleotides 494–522 and 932–961 of the rat H_{3L}-receptor sequence for amplification in rat, or primers 5 and 6 corresponding to nucleotides 1–33 of the rat H_3-receptor coding sequence and nucleotides 1,601–1,637 of the human H_3-receptor sequence[13] for amplification in mouse. Rat PCR products were electrophoresed, blotted overnight onto nylon membranes, and hybridized with the ^{32}P-labelled 607-base-pair cDNA probe described above. Mouse PCR products were subcloned into pGEM-T Easy plasmid (Promega) and sequenced.

Stable transfection of CHO-K1 cells

The cDNA inserts corresponding to the full-length coding sequence of the H_{3S} and H_{3L} isoforms were ligated into the mammalian expression vector pCIneo (Promega). CHO-K1 cells were transfected with SuperFect reagent (Qiagen). Stable transfectants were selected with 2 mg ml^{-1} G418 and tested for [^{125}I]iodoproxyfan binding[22]. Several clones, named CHO(H_{3S}) and CHO(H_{3L}), expressing various receptor densities, were selected for further characterization and maintained in the presence of 1 mg ml^{-1} G418.

[^{125}I]iodoproxyfan-binding assay

CHO (H_{3L} or H_{3S}) cells were washed and homogenized (Polytron) in ice-cold binding buffer (Na_2HPO_4/KH_2PO_4 50 mM, pH 6.8), and binding assays were done as described[22].

cAMP accumulation

CHO (H_{3L} or H_{3S}) cells (96-well plates) were incubated for 10 min at 37 °C with 3 μM forskolin and, when required, 1 μM thioperamide in DMEM/Nut mix F-12 containing 100 μM isobutylmethylxanthine. cAMP was extracted and measured by

radioimmunoassay (NEN Life Science Products). We carried out statistical evaluation of the results by analysis of variance (ANOVA) followed by Newman–Keuls test.

[^{3}H]arachidonic acid release

CHO (H_{1L} or H_{1S}) cells (24-well plates) were incubated for 2 h at 37 °C with 0.5 μCi of [^{3}H]arachidonic acid in DMEM/Nut mix F12 containing 0.2% bovine serum albumin. After washings, cells were incubated for 30 min with 2 μM A23187 and, when required, the H_3-receptor ligands and [^{3}H]arachidonic acid release was determined by liquid scintillation counting. We carried out statistical evaluation of the results by ANOVA followed by Newman–Keuls test.

[^{3}H]histamine release from synaptosomes

After a 30-min pre-incubation of mouse or rat cortical synaptosomes with [^{3}H]L-histidine, [^{3}H]histamine release induced by potassium was evaluated[26]. Total [^{3}H]histamine initially present in synaptosomes represented about 3,500 d.p.m per mg protein. For reversal of histamine-induced inhibition of [^{3}H]histamine release, drugs in increasing concentrations were opposed to 1 μM histamine in the presence of 30 mM K^+. The effect of thioperamide (100 nM) was studied on [^{3}H]histamine release induced by 20–55 mM K^+. The effects of various H_3-receptor ligands (100 nM) were studied on [^{3}H]histamine release induced by 55 mM K^+ in the presence or absence of 10 μM proxyfan. Statistical evaluation of the results was performed using ANOVA followed by Newman–Keuls test.

Histamine and [^{35}S]GTPγS-binding assays

We measured histamine in the medium of depolarized mouse cortical synaptosomes and of cell cultures using an enzymoimmunoassay (Immunotech, Marseille, France). For [^{35}S]GTPγS binding assays, mouse or rat cerebral cortical membranes were pretreated with adenosine deaminase (1 U ml^{-1}) and incubated as described[27] for 30 min at 25 °C with 0.1 nM [^{35}S]GTPγS and various H_3-receptor ligands (10 nM) in the presence or absence of 1 μM proxyfan. Statistical evaluation of the results was performed using ANOVA followed by Newman–Keuls test.

Assay of *tele*-methylhistamine (t-MeHA) in brain

Drugs were administered p.o. to male Swiss mice (Iffa-Credo, L'Arbresle, France) and brain t-MeHA levels were determined after 90 min by enzymoimmunoassay[24]. Results are expressed as per cent change compared with values obtained in vehicle-treated mice (119 ± 4 ng per g).

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Attenuation of FGF signalling in mouse β-cells leads to diabetes

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Fibroblast growth factor (FGF) signalling has been implicated in patterning, proliferation and cell differentiation in many organs, including the developing pancreas[1,2]. Here we show that the FGF receptors (FGFRs) 1 and 2, together with the ligands FGF1, FGF2, FGF4, FGF5, FGF7 and FGF10, are expressed in adult mouse β-cells, indicating that FGF signalling may have a role in differentiated β-cells. When we perturbed signalling by expressing dominant-negative forms of the receptors, FGFR1c and FGFR2b, in the pancreas, we found that that mice with attenuated FGFR1c signalling, but not those with reduced FGFR2b signalling, develop diabetes with age and exhibit a decreased number of β-cells, impaired expression of glucose transporter 2 and increased proinsulin content in β-cells owing to impaired expression of prohormone convertases 1/3 and 2. These defects are all characteristic of patients with type-2 diabetes. Mutations in the homeobox gene *Ipf1/Pdx1* are linked to diabetes in both mouse and human. We also show that *Ipf1/Pdx1* is required for the expression of FGFR1 signalling components in β-cells, indicating that *Ipf1/Pdx1* acts upstream of FGFR1 signalling in β-cells to maintain proper glucose sensing, insulin processing and glucose homeostasis.

Pancreatic β-cell dysfunction is a critical component in the development of type-2 diabetes. The disease results in part from an inability of β-cells to produce and secrete sufficient amounts of active insulin in response to an increased demand for insulin[3,4]. To decipher the molecular machinery of β-cell function, we first investigated whether extrinsic factors, such as FGF signalling factors, are involved in the differentiation of pancreatic β-cells. We analysed the expression of FGFR1 and FGFR2 in the adult mouse pancreas. FGFR1 and FGFR2 were both expressed predominantly in β-cells, with no expression observed in glucagon-producing α-cells (Fig. 1a, d). A low level of FGFR1 expression was also observed in cells of the exocrine pancreas, whereas no expression